Current Topics in Genome Analysis

Cytogenetic Methods

January 26, 1999

Thomas Ried, M.D.
Genetics Department
National Cancer Institute/NIH
e.mail: tried@nhgri.nih.gov

Fluorescence in situ hybridization (FISH)

DNA in situ hybridization is a technique that allows the visualization of defined sequences of nucleic acids at the cellular and subcellular level. The method is based on the site specific annealing (hybridization) of single-stranded DNA molecules (probes) to denatured, complementary sequences (targets) on cytological preparations, like metaphase chromosomes or interphase nuclei. After fluorescence detection steps, the probe sequences become visible at the site of hybridization.

FISH is a multistep procedure. Specific protocols for each of the steps are provided in Part II. The single steps are, simplified, summarized as follows.

1. Preparation of DNA probes
2. Labeling of DNA probes
3. Preparation of cytological specimens
4. Denaturation of probe and specimen
5. In situ hybridization
6. Fluorescence probe detection
7. Fluorescence microscopy

1. DNA preparation usually follows standard procedures. Modifications are described in detail in Part II.

2. The labeling of DNA or RNA probes for FISH is generally performed enzymatically. Even though chemical procedures are available, the enzymatic protocols using Nick-translation, random priming, PCR or tailing with terminal transferase proved to be the simplest and most reliable labeling protocols. During the labeling reaction modified nucleotide analogs are incorporated. They are linked to haptens, e.g., biotin, digoxigenin, or Dinitrophenol. Recently, nucleotide analogs became available, that are directly conjugated to fluorochromes (Wiegant et al., 1991), such as FITC-dUTP or TRITC-dUTP.

3. The preparation of metaphase or prophase chromosomes for in situ hybridization follows standard cytogenetic protocols (Verma and Babu, 1989). FISH can be easily combined with chromosome banding protocols (Arnold et al., 1992). Interphase nuclei, e.g., amniotic fluid cells, fibroblasts, or nuclei in tissue sections need various pretreatment steps in order to increase probe accessibility and to reduce fluorescence background staining.

4. The probe molecules and the target DNA are denatured thermally. Formamide is added to reduce the melting temperature of the double stranded DNA. If complex DNA probes are used, an additional preannealing step with an excess of unlabeled total genomic DNA or the Cot1-fraction of human DNA prior to the hybridization is required, leading to the term chromosomal in situ suppression (CISS) hybridization (Cremer et al., 1988).

5. The hybridization reaction is usually carried out at 37°C for about 16 hours. Shorter hybridization

times (minutes to hours) are sufficient for probes that detect repetitive sequence motifs. Certain probes require increased hybridization temperatures in order to exclusively label their target region. If entire genomes are hybridized, e.g. using comparative genomic hybridization (CGH), prolonged hybridization times are necessary.

6. The detection reaction is performed indirectly with fluorochromes linked to avidin or antibodies against the reporter molecules. If probes are used that are conjugated with fluorochromes, detection steps are not required. Numerous fluorochromes are available including fluorochromes emitting in the blue (AMCA, Cascade blue), in the green (FITC), and in the red (TRITC, Texas red, rhodamine, Cy3). More recently, fluorochromes which emit in the infrared, such as Ultralite 680 or Cy5, became commercially available.

7. Probe signals are visualized by epifluorescence microscopy. New generations of specific filter sets allow to precisely separate the fluorochromes (Ploem and Tanke, 1987; Marcus, 1988). A convenient development, in particular with respect to the needs of routine diagnostic laboratories provide double or triple band pass filters (Johnson et al., 1991). They are used to simultaneously visualize several fluorochromes. Digital imaging devices with a high photon detection efficiency and a high dynamic range, like silicon intensified tube cameras or charge coupled device (CCD) cameras add significantly to the sensitivity and provide the basis to quantify fluorescence images (Hiraoka et al., 1987). CCD cameras are also sensitive over a broad spectral range, thus fluorochromes emitting in the infrared spectrum can be included as fluorescence detection systems. Confocal laser scanning microscopy is preferred if light optical sectioning of three dimensional specimens, like interphase nuclei, is desired (Cremer and Cremer, 1978).

Complementing each other, these developments have contributed to the tremendous improvements of FISH over the last few years with respect to sensitivity, resolution, and multiplicity. DNA or cDNA probes as small as 500 bp can be visualized on metaphase chromosomes. This equals the sensitivity of isotopic detection formats. The spatial resolution of fluorescence signals is higher than the one involving radioisotopes and is, on metaphase chromosomes in the range of some 5 Mbp. However, the less condensed interphase chromatin increases the resolution to approximately 100 kbp (Lawrence et al., 1990; Trask et al., 1991). Recently, several techniques for extended chromatin preparations have improved the resolution power dramatically (Heng et al., 1992; Wiegant et al., 1992; Parra and Windle, 1993). Using histone depleted interphase nuclei and other high resolution FISH techniques, the spatial resolution is in the range of 5 kbp (Tocharoentanaphol et al., 1994). The improvements of sensitivity and spatial resolution have had considerable impact on gene mapping and studies dealing with the 3D organization of chromatin in interphase nuclei (Florijn et al., 1995; Heiskanen et al., 1996; Michalet et al., 1997).

The possibility of visualizing several chromosomal targets simultaneously has broadened

the spectrum of diagnostic and research applications of FISH and has become one of the most attractive features of FISH analysis. The number of suitable labeling and detection formats still limits the multiplicity of FISH. To overcome these limitations, approaches using combinatorial or ratio labeling of single probes have been devised. They increase the number of target regions that can be discerned by means of their respective color after a single hybridization experiment beyond the number of available fluorochromes (Nederlof et al., 1990; Ried et al., 1992a,b; Dauwerse et al., 1992; Wiegant et al., 1993; Lengauer et al., 1993). For example, with three labeling and detection systems, a total of seven probes can be distinguished. Probes 1, 2, and 3 would be visualized as a pure fluorochrome, while probes 4-7 would appear as fluorochrome mixtures as follows: probe 4, FITC and TRITC; probe 5, FITC and AMCA; probe 6, TRITC and AMCA; and probe 7, FITC, TRITC, and AMCA. Using digital image analysis fluorochromes emitting in the infrared can be included. Thus, DAPI might be used to counterstain the chromosomes which gives additional information. The possibility to use digital imaging devices for an accurate quantification of FISH signals also forms the basis for the newly introduced technique of comparative genomic hybridization (CGH).

An equally important improvement for the application of FISH in medical diagnosis is the availability of different probe sets. This increases the flexibility to design experimental protocols to specifically address the diagnostic requirements. One of the first probes used for FISH analysis comprised cloned DNA fragments that contained consensus sequences for the repeat units of centromeric or paracentromeric heterochromatin of specific chromosomes. Using appropriate stringency conditions the centromeric region of almost every human chromosome can be visualized specifically (Willard and Waye, 1987; Vogt, 1990). The limitations of these probes, however, are obvious. Since all chromosome specific repetitive DNA reported to date is localized to discrete subregions of each chromosome, this class of DNA probes is unsuitable for the analysis of many types of chromosomal aberrations, e.g. translocations and deletions involving chromosomal arms. Their use is, therefore, with few exceptions restricted to the assessment of numerical aberrations.

These limitations were overcome with the advent of composite probe sets specific for entire chromosomes, also termed chromosome painting probes (Pinkel et al., 1988; Cremer et al., 1988; Lichter et al., 1988). Based on the enrichment of individual chromosomes by means of flow cytometry, fragments of isolated chromosomes were cloned in phage and plasmid vectors (Collins et al., 1991). Since genomic DNA clones do not only contain chromosome specific single copy sequences, but also highly repetitive elements of the SINE and LINE families (for review see, e.g., Vogt, 1990), the successful delineation of individual chromosomes depends on the use of suppression hybridization protocols (Cremer et al., 1988). An excess of unlabeled DNA derived from the Cot1-fraction of human DNA is used to block the cross hybridization of ubiquitously distributed repetitive DNA fragments.

The rapid progress of DNA cloning technology and the success of the Human Genome Project made an increasing number of region or gene specific DNA clones available that can be used to pinpoint specifically the variety of chromosomal aberrations involved in human genetic diseases. In complementation to these developments, efficient protocols became available in order to selectively enrich the human DNA content in a background of e.g. hamster (Lengauer et al., 1990) or yeast DNA (Nelson et al., 1989; Lengauer et al., 1992a).

Rather recently developed probe generations include region specific probes derived from microdissected human chromosomes (Lengauer et al., 1991; Meltzer et al., 1992, Guan et al., 1994) and probes generated from cytogenetically detected marker chromosomes, a procedure termed "reverse painting" (Carter et al., 1992). Finally, entire genomes can be used as probes (Kallioniemi et al., 1992; Kallioniemi et al., 1993; du Manoir et al., 1993; Speicher et al., 1993, Schröck et al., 1994, Ried et al., 1994). In a comparative hybridization format, these probes are used to reveal partial or complete chromosome gains and losses in test genomes, e.g., in DNA extracted from solid tumor cells.

Since the first report on in situ hybridization protocols by Gall and Pardue (1969), FISH has evolved as a powerful and versatile experimental tool in genetic research. In basic research, FISH contributed to the understanding of nuclear topography, both of mammalian and plant cells. The experimental evidence, established after UV-laser microirradiation of interphase nuclei, that chromosomes are organized as discrete territories in the cell nucleus (Cremer et al., 1982a,b) was confirmed elegantly using DNA from hybrid cell lines (Manuelidis, 1985; Schardin et al., 1985) or cloned DNA libraries from individual chromosomes as probes (Cremer et al., 1988; Lichter et al., 1988a; Pinkel et al., 1988). The distribution of chromosome centromeres was investigated by FISH in interphase cells of tissue sections and isolated nuclei, and provided evidence for a non-random, cell type specific arrangement (Manuelidis, 1984; Arnoldus et al., 1989; Haaf and Schmid, 1989; van Dekken et al., 1990; Popp et al., 1990; Arnoldus et al., 1991; Weimer et al., 1992). Furthermore, taking advantage of confocal laser scanning microscopy, subchromosomal compartments were defined and the distribution of certain genes with respect to the chromosome domain was successfully investigated (Spector, 1990; Spector et al., 1991; Zirbel et al., 1993; Cremer et al., 1994). By means of RNA in situ hybridization, a distinct compartmentalization of transcriptional mechanisms was determined (Lawrence et al., 1989; Carter et al., 1991; Carter et al., 1993; Xing et al., 1993).

Fluorescence in situ hybridization to metaphase chromosome preparations revealed distinct structural features of the arrangement of repetitive DNA sequences, as well as the nonrandom distribution of genes with respect to chromosome bands (Korenberg and Rykowski, 1988; Chen and Manuelidis, 1989; Holmquist, 1992). Chromatin packaging models were assayed by FISH with DNA clones for specific genes and revealed a fixed lateral position on metaphase chromosomes (Baumgartner et al., 1991). Selig et al. (1992) used FISH to monitor replication timing in a series of

different cell types, and mapped the replication timing topography of the cystic fibrosis locus. Another study revealed an allele-specific replication timing (Kitsberg et al., 1993).

FISH to meiotic chromosomes of human and mouse origin was used to track down basic events in meiosis, such as nondisjunction and recombination (Pieters et al., 1990; Guttenbach and Schmid, 1991; Ashley et al., 1994).

Comparative mapping studies with human DNA probes to chromosomes from great apes, hylobatids, old world monkeys and prosimians, established a molecular taxonomy. As yet unidentified chromosomal rearrangements that occured during the course of mammalian chromosome evolution were delineated with high resolution. Thus, entire karyotypes were reconstructed with chromosome specific DNA libraries and region specific DNA probes (Wienberg et al., 1990; Wienberg et al., 1992; Jauch et al., 1992; Ried et al., 1993a).

Based on suppression hybridization with cosmid and YAC clones (Landegent et al., 1987), FISH was introduced as an important method to the international efforts of the human genome project. Large numbers of DNA clones could be mapped on human metaphase and prometaphase chromosomes by means of fractional-length measurements (Lichter et al., 1990a) or, combined with cytogenetic banding techniques (Klever et al., 1991; Baldini and Ward, 1991; Arnold et al., 1992) with respect to chromosome bands (Ward et al., 1991; Bellanné-Chantelot et al., 1992; Cohen et al., 1993). The hybridization of low complexity cDNA clones made it also possible to rapidly assess the chromosomal location of candidate disease genes, and to compare these mapping positions with data from, e.g., genetic linkage studies based on pedigree analysis (Ried et al., 1993c).

The application of FISH to problems in medical diagnosis are numerous (for a review see, e.g., Tkachuk et al., 1991): in clinical cytogenetics, FISH analysis is often a helpful adjunct to chromosome banding studies, and is used to confirm, or in some cases even to allow to determine the origin of marker chromosomes and to highlight numerical and structural aberrations (Jauch et al., 1990; Popp et al., 1993). In general, FISH has the distinct advantage that the diagnosis of numerical and structural chromosomal aberrations is not restricted to dividing cells, i.e., metaphase chromosomes, but is applicable during all stages of the cell cycle, a feature termed interphase cytogenetics (Cremer et al., 1986). Interphase cytogenetics has become an useful diagnostic tool in cancer cytogenetics (Nederlof et al., 1989; Tkachuk et al., 1990; Ried et al., 1992c; Lengauer et al., 1992a; Ried et al., 1993b). Also, the diagnosis of trisomy 21 is performed in many laboratories on a routine basis directly in interphase nuclei (Klinger et al., 1992; Klever et al., 1992; Ward et al., 1993). The high resolution of FISH analysis allows for a sensitive visualization of even submicroscopical deletions. This has implications for the diagnosis of constitutional microdeletion syndromes (Ledbetter, 1992a), the diagnosis of carrier status in X-chromosomal recessively inherited diseases associated with deletions (Ried et al., 1990), and the identification of deletions of tumor suppressor genes in certain types of human malignancies (Stilgenbauer et al., 1993). Pathogen

based diagnostic procedures, such as the detection of virus genomes in tissue sections have been reported (Brigati et al., 1983). The development of biological dosimeters for a follow up and long term screening of individuals who were exposed to radiation, is an useful tool to determine the effects of ionizing substances, resulting in dicentric chromosomes and translocations (Cremer et al., 1990; Popp and Cremer, 1992; Gray et al., 1992).

1. Methodological introduction to comparative genomic hybridization (CGH)

Comparative genomic hybridization is a new molecular cytogenetic technique based on quantitative two color fluorescence in situ hybridization (Kallioniemi et al., 1992; Kallioniemi et al., 1993; du Manoir et al., 1993). CGH allows, in a single experiment, to detect genetic imbalances in solid tumors or any desired test genome, and to determine the chromosomal map position of gains and losses of chromosomes or chromosomal subregions on normal reference metaphase preparations. Total genomic DNA from a tumor specimen is isolated following standard procedures. A reference, or control DNA is isolated from an individual with a normal karyotype (46,XX or 46,XY). DNA extracted from non-involved tissue of a tumor patient can be used as well as reference DNA. The two genomes are labeled differentially with reporter molecules (e.g., biotin-dUTP for the tumor genome, and digoxigenin-dUTP for the reference genome) in a standard nick translation reaction. The so labeled genomes are then pooled and hybridized to reference human metaphase spreads (46,XY). In order to reduce the cross hybridization of highly repetitive sequences present in both genomes, an excess of unlabeled Cot1-fraction of human DNA is included in the hybridization mixture. This step is necessary because the high hybridization kinetics of repetitive DNA might impair the evaluation of the single copy sequences that are over- or underrepresented in the tumor genome. In a subsequent step, the hybridized probes (genomes) are visualized with different fluorochromes (e.g. avidin-FITC, green fluorescence, for the biotinylated tumor genome and anti-digoxigenin coupled to rhodamine, red fluorescence, for the reference genome). The differences in fluorescence intensities along the chromosomes on the reference metaphase spread reflect the copy number of corresponding sequences in the tumor DNA. If chromosomes or chromosomal subregions are present in identical copy numbers in both, the reference and the tumor genome, the observed fluorescence is a blend of an equal contribution of red and green fluorescence. If chromosomes are lost or chromosomal subregions deleted in the tumor genome, the resulting color is shifted to red. A gain of a certain chromosome in the tumor would be reflected by a more intense green staining on the respective chromosome in the reference metaphase preparation. Increased supernumerary, e.g. local DNA amplification results in green signals of similar shape and intensity as single copy probes, e.g., YAC clones.

In many instances, gross chromosomal aberrations in tumor genomes, such as high

level DNA amplifications, are visible directly in the fluorescence microscope. However, a quantitative measurement of fluorescence intensity values based on digital image analysis is crucial for a precise CGH analysis of low copy number changes. This analysis includes image acquisition of the rhodamine and FITC fluorescence with a CCD camera. Using custom computer software, the painted chromosomes are then segmentated and the fluorescence values determined perpendicular to the axis of the chromosome on a pixel to pixel basis. The result of the measurement of the fluorescence values can now be visualized by means of a look up table where certain colors refer to gains or losses in the tumor genome. The final step in a quantitative fluorescence measurement includes the calculation of average ratio profiles along the chromosomal axis based on data from at least 5 metaphase spreads. Values of 1 indicate equal copy numbers of the respective chromosomes in the tumor and test genome, a ratio of 0.5 a deletion of one homologous chromosome and ratios of 1.5 reflect a trisomy in the tumor. Gene amplifications can be mapped to reference metaphase chromosomes according to peak fluorescence ratios of more than 2.5. For a detailed description of the CGH-software the reader is referred to du Manoir et al., (1995).

The validity of CGH to delineate complex genetic changes in solid tumors has been investigated in several studies. Using a cell line established from a renal cell carcinoma, the results from karyotype analysis were compared with CGH. All chromosomal aberrations detected after karyotyping could be confirmed after the CGH analysis (Du Manoir et al., 1993). Another, independent study to verify the results of CGH analysis was described by Schröck et al. (1994) with a series of human gliomas. In this sample collection, banding was often impossible due to inferior spreading of the metaphase chromosomes and the frequent observation of DM chromosomes. By means of interphase cytogenetics with YAC clones for chromosomal subregions that revealed gains and losses after CGH, the presence of all imbalances could be confirmed in interphase nuclei prepared from tissue sections, i.e., ratios of 1.5 after CGH were in accordance with three signals in interphase nuclei. In addition, a DNA amplification that was mapped to chromosome 4 by CGH, was shown to be present in DM chromosomes of this tumor after FISH with a chromosome 4 specific DNA library to metaphase chromosome preparations. Also, the amplification of the EGFR-gene determined by DNA fingerprint analysis resulted in peak fluorescence values on chromosomal map position 7p12, known to harbor the gene encoding this growth factor receptor.

One of the distinct advantages of CGH is the fact that tumor DNA is the only requirement for this molecular cytogenetic analysis. Thus, archived, formalin fixed and paraffin embedded tissue can be used as well (Speicher et al., 1993). This allows to establish a correlation of the microscopic phenotype and the genotype in solid tumors (Speicher et al., 1995). In combination with microdissection of distinct areas on microscopically defined tissue sections, CGH offers a new experimental approach to study chromosomal aberrations that occur during solid tumor progression (e.g., Ried et al., 1995; Schröck et al., 1996; Ried et al., 1996; Heselmeyer et al., 1996).

CGH-technique and applications have been reviewed in the following manuscripts: Forozan et al., (1997); Ried et al., (1997); Knuutila et al., 1998).

2. Introduction to spectral imaging and spectral karyotyping (SKY)

With all the advantages and the particular elegance of comparative genomic hybridization one should not overlook its limitations. CGH allows one to identify only those chromosomal aberrations that result in DNA copy number changes. For instance, a chromosomal aberration such as the Philadelphia chromosome -arguably an important event in the transformation of hematological cells of myeloid origin, would remain undetected by CGH. Also, the chromosomal mechanisms by which individual cells generate copy number changes, e.g., duplications, isochromosome formations, dmins, hsr's, and others, remain elusive. And lastly, at the present stage of technology development, CGH generates an average of the most common aberrations in tumor genomes, disregarding important features such as clonal heterogeneity, which provides tumors with the genetic diversity to react more flexibly to environmental changes. FISH, using the plethora of available probe sets is an important technique to analyze chromosomal aberrations on a single cell level. However, a targeted analysis of, e.g., the deletion status of a tumor suppressor gene, leaves the rest of the genome unanalyzed. Therefore, the cytogeneticist would like to add to the methodological spectrum an approach that allows to visualize all human chromosomes in different colors. The goal to increase the number of chromosomal targets that can be discerned simultaneously, i.e., the multiplicity of FISH experiments has long been perceived (Nederlof et al., 1990; Ried et al., 1992). The scarcity of suitable probe labeling and fluorescence detection formats, however, makes this a non-trivial task. This is mainly due to the nature of the fluorochromes itself. In many instances, the emission spectra of fluorochromes overlap. Therefore it is difficult to discern an ever increasing number of fluorochromes using conventional, fluorochrome specific optical filters, and color karyotyping was not possible until recently when Speicher and colleagues reported the FISH-based discernment of all human chromosome using sequential exposures with 6 different optical filters (Speicher et al., 1996). We have developed a novel approach for the visualization of FISH experiments. In strong contrast to conventional epifluorescence filter technology, we have explored the possibility of using spectral imaging to distinguish multiple and overlapping fluorochromes simultaneously, and hence achieved the goal of color karyotyping human (and other species) chromosomes (Schröck et al., 1996; Liyanage et al., 1996).

Methodology

Spectral imaging refers to a novel imaging technique for the analysis of FISH experiments (Schröck et al., 1996). The application to karyotype analysis is termed spectral karyotyping (SKY) and is based on spectral imaging (Malik et al., 1996; Garini et al., 1996). Spectral imaging, as the terminology suggests, combines spectroscopy and imaging. In dramatic contrast to conventional epifluorescence microscopy in which fluorochrome discrimination is based on the measurement of a single intensity through a fluorochrome specific optical filter, spectral imaging allows to measure and analyze the full spectrum of light at all pixels of the image. The light emitted from each point of the sample is collected with the microscope objective and sent to a collimating lens (the system can also be attached to any other optics like a telescope or a simple lens). The collimated light travels through a Sagnac interferometer and is focused on a CCD. The data is collected and processed with a personal computer. The heart of a spectral imaging system consists of an optical dispersion element that allows measurement of the full spectrum for each pixel (Malik et al., 1996).

The spectral imaging system is based on the SD 200 SpectraCube-technology. The approach described here is based on Fourier spectroscopy (Bell, 1972). This method offers several advantages compared to other methods for spectral imaging, such as accusto-optical tunable filters. Most importantly, the spectral imaging system has a high optical throughput, high (and variable) spectral resolution, a broad spectral band-width, and it is independent on polarization.

Measuring the full spectrum for each pixel has major advantages over measurements of one or few gray level images through specific filter cubes. The most important advantages are:

- Emission spectra of all fluorochromes are measured in a single exposure. Consequently, image registration problems do not exist, and all information is contained in one spectral image.
- Distinction of fluorochromes with overlapping emission spectra even if spectral shifts are subtle.
- The analysis is not based on absolute intensities not sensitive to changes of the intensity of one or few of the fluorochromes. Although this information exists in the spectral image, the analysis is based only on differences in spectral shapes of the different fluorochromes.
- The measurement of the entire spectrum allows to readily change a set of dyes. This provides flexibility to integrate new fluorescence dyes.
- Background fluorescence can be precisely measured, and, therefore, subtracted. This increases the accuracy of fluorochrome (and therefore chromosome) identification.
- Autofluorescence of biological structures can be identified and subtracted, because, in general, the spectrum should be different from the signal's spectrum.

The application of spectral imaging to the field of cytogenetic research and diagnostics is

termed spectral karyotyping, or SKY. In the following we will describe some of these applications to chromosome analysis both in human malignancies as well as in animal models of certain tumors, and we will try to paint a picture on how cytogenetic diagnostics might be performed in the near future.

Application of spectral karyotyping

Spectral karyotyping of human chromosomes is based on the simultaneous hybridization of a 24 chromosome specific probe pool. Chromosome specific probe pools, or chromosome painting probes, can be generated from flow-sorted human chromosomes (Telenius et al., 1992) or by chromosome microdissection (Guan et al., 1994). In order to produce a chromosome specific spectrum after hybridization, each chromosome library was labeled either with a single fluorochrome or with specific combinations of two or three fluorochromes, allowing us to increase the number of discernible targets beyond the number of fluorochromes that are suitable for DNA-labeling. Using combinatorial labeling with five different fluorochromes, 31 different targets can be distinguished. The hybridization was visualized using spectral imaging through a single optical filter that allowed for the excitation of all fluorochromes, and the measurement of their emission spectra without the need to change from one fluorochrome specific optical filter to another. The applications of SKY to visualize chromosomal aberrations involved in human diseases are manifold. Chromosome banding based karyotype analysis is routinely performed in the prenatal and postnatal cytogenetic laboratory. The benefits of SKY in this field include (i) the identification of subtle chromosomal aberrations such as the translocation of telomeric chromatin that is difficult to detect using banding alone and (ii) the identification of small markers that remain elusive after banding. In a recently conducted study of cases with unidentified constitutional chromosome abnormalities SKY was able to refine karyotype interpretation in the majority of the cases (Schröck et al., 1997). SKY, in combination with chromosome banding analysis might also enable the automation of karyotype analysis in the clinical cytogenetic laboratory where the majority of the karyotypes are actually normal. However, the need to complement karyotype analysis with SKY is even more obvious in tumor cytogenetics. This is due to certain, characteristic features of metaphase chromosomes from malignant cells. In many instances the mitotic index is low. As a consequence, the few cells that are available would preferably be analyzed as comprehensively as possible. Also, tumor metaphase preparations, in particular those established from solid tumors and lymphomas are often of poor quality which precludes high resolution banding analysis. The matter becomes even more complicated because tumor karyotypes are often highly rearranged. This shuffling of chromosomal segments makes it extremely difficult to identify the origin of translocated chromatin because the sequence of

chromosomal bands is obscured. This problem could be overcome by adding color information that unambiguously identifies the origin of rearranged chromosomal material. Indeed, it has been shown that the combination of banding and SKY allows one to identify marker chromosomes and also chromosomal breakpoints with higher accuracy than in the past (Veldman et al., 1997). Lastly, SKY was successfully used to characterize chromosomal structures such as dmin's, hsr's, and other cytogenetic reflections of oncogene amplification whose origin could not be deduced by banding methods (Macville et al., 1999; Sawyer et al., 1998).

Literature

Arnold N, Bhatt M, Ried T, Wienberg J, Ward DC (1992) Fluorescence in situ hybridization on banded chromosomes. In: Techniques and methods in molecular biology: nonradioactive labeling and detection of biomolecules. Kessler C (ed) Springer Verlag, Heidelberg, New York.

Arnoldus EPJ, Peters ACB, Bots GTAM, Raap AK, van der Ploeg M (1989) Somatic pairing of chromosome 1 centromeres in interphase nuclei of human cerebellum. Hum Genet 83:231-234.

Arnoldus EPJ, Noordermeer IA, Peters ACB, Raap AK, van der Ploeg M (1991) Interphase cytogenetics reveals somatic pairing of chromosome 17 centromeres in normal human brain tissue, but no trisomy 7 or sex-chromosome loss. Cytogenet Cell Genet 56:214-216.

Ashley T, Ried T, Ward DC (1994) Detection of nondisjunction and recombination in meiotic and post meiotic cells from $XY,_{Sxr}$ mice using multicolor FISH. Proc Natl Acad Sci USA 91:524-528.

Baldini A, Ward DC (1991) In situ hybridization of human chromosomes with Alu-PCR products: a simultaneous karyotype for gene mapping studies. Genomics 9:770-774.

Bauman JGJ, Bentvelzen P (1988) Flow cytometric detection of ribosomal RNA in suspended cells by fluorescent in situ hybridization. Cytometry 9: 517-524.

Baumgartner M, Dutrillaux B, Lemieux N, Lilienbaum A, Paulin D, Viegas-Péquignot E (1991) Genes occupy a fixed and symmetrical position on sister chromatids. Cell 64:761-766.

Bell RJ (1972) Introductory Fourier transform spectroscopy. Academic Press, London.

Bellanné-Chantelot C, Lacroix B, Ougen P, Billaut A, Beaufils S, Betrand S, Georges I, Glibert F, Gros I, Lucotte G, Susini L, Codani JJ, Gesnouin P, Pook S, Vaysseix G, Lu-Kuo J, Ried T, Ward DC, Chumakov I, Le Paslier D, Barillot E, Cohen D (1992) Mapping the whole human genome by fingerprinting yeast artificial chromosomes. Cell 70:1059-1068.

Beverloo HB, van Schadewijk A, Van Gelderen-Boele S, Tanke HJ (1990) Inorganic phosphors as newluminescent labels for immunocytochemistry and time-resolved microscopy. Cytometry 11:84-792.

Bohlander SK, Espinosa R, Le Beau MM, Rowley JD, Diaz MO (1992) A method for the rapid sequence-independent amplification of microdissected chromosome material. Genomics 13:1322-1324.

Brigati DJ, Myerson D, Leary JJ, Spalholz B, Travis SZ, Fong CKY, Hsuing GD, Ward DC (1983) Detection of viral genomes in cultured cells and paraffin-embedded tissue sections using biotin-labeled hybridization probes. Virology 126:32-50.

Carter KC, Taneja KL, Lawrence JB (1991) Discrete nuclear domains of poly(A) RNA and their relationship to the functional organization of the nucleus. J Cell Biol 115:1191.

Carter KC, Bowman D, Carrington W, Fogarty K, McNeil JA, Fay FS, Lawrence JB (1993) A three dimensional view of precursor messenger RNA metabolism within the mammalian nucleus. Science 259:1330-1335.

Carter NP, Ferguson-Smith MA, Pmyman MT, Telenius H, Palmear AH, Leversha MA, Glancy MT, Wood SL, Cook K, Dyson HM, Ferguson-Smith ME, Willat R (1992) Reverse chromosome painting: a method for the rapid analysis

of aberrant chromosomes in clinical cytogenetics. J Med Genet 29:299-307.

Chen TL, Manuelidis L (1989) SINEs and LINEs cluster in disctinct DNA fragments of Giemsa band size. Chromosoma 98:309-316.

Cohen D, Chumakov I, Weissenbach J (1993) A first-generation physical map of the human genome. Nature 366:698-701.

Collins CC, Kuo WL, Segraves R, Fuscoe JC, Pinkel D, Gray JW (1991) Construction and characterization of plasmid libraries enriched in sequences from single human chromosomes. Genomics 11:997-1006.

Cremer C, Cremer T (1978) Considerations on a laser-scanning microscope with high resolution and depth of field. Microscopia Acta 81:31-44.

Cremer T, Cremer C, Baumann H, Luedtke EK, Sperling K, Teuber V, Zorn C (1982a) Rabl's model of the interphase chromosome arrangement tested in chinese hamster cells by premature chromosome condensation and laser-UV-microbeam experiments. Hum Genet 60:46-56.

Cremer T, Cremer C, Schneider T, Baumann H, Hens L, Kirsch-Volders M (1982b) Analysis of chromosome positions in the interphase nucleus of chinese hamster cells by laser-UV-microirradiation experiments. Hum Genet 62:201-209.

Cremer T, Landegent JE, Brueckner A, Scholl HP, Schardin M, Hager H-D, Devilee P, Pearson PL, van der Ploeg M (1986) Detection of chromosome aberrations in the human interphase nucleus by visualization of specific target DNAs with radioactive and nonradioactive in situ hybridization techniques. Hum Genet 74:346-352.

Cremer T, Lichter P, Borden J, Ward DC, Manuelidis L (1988) Detection of chromosome aberrations in metaphase and interphase tumor cells by in situ hybridization using chromosome specific library probes. Hum Genet 80:235-246.

Cremer T, Popp S, Emmerich P, Lichter P, Cremer C (1990) Rapid metaphase and interphase detection of radiation induced chromosome aberrations in human lymphocytes by chromosomal suppression in situ hybridization. Cytometry 11:110-118.

Cremer T, Kurz A, Zirbel R, Dietzel S, Rinke B, Schröck E, Speicher MR, Mathieu U, Jauch A, Emmerich P, Scherthan H, Ried T, Cremer C, Lichter P (1993) The role of chromosome territories in the functional compartmentalization of the cell nucleus. Cold Spring Harbor Symp Quant Biol vol. LVIII. p. 777-792. Cold Spring Harbor, New York.

Dauwerse JG, Wiegant J, Raap AK, Breuning MH, van Ommen GJB (1992) Multiple colors by fluorescence in situ hybridization using ratio-labelled DNA probes create a molecular karyotype. Hum Mol Genet 1:593-598.

Du Manoir S, Speicher M.R, Joos S, Schröck E, Popp S, Döhner H, Kovacs G, Robert-Nicoud M, Lichter P, Cremer T (1993) Detection of complete and partial chromosome gains and losses by comparative genomic in situ hybridization. Hum Genet 90:590-610.

Du Manoir S, Schröck E, Bentz M, Speicher MR, Joos S, Ried T, Lichter P, Cremer T. (1995) Quantitation of comparative genomic hybridization. Cytometry 19:27-41.

Florijn RJ, Bonden LA, Vrolijk H, Wiegant J, Vaandrager JW, Baas F, den Dunnen JT, Tanke HJ, van Ommen GJ, Raap AK. High-resolution DNA Fiber-FISH for genomic DNA mapping and colour bar-coding of large genes. Hum Mol Genet 1995;4:831-6

Forozan F, Karhu R, Kononen J, Kallioniemi A, Kalioniemi O-P: Genome screening by comparative genomic hybridization. TIG 1997, 13:405-409

Garini Y, Katzir N, Cabib D, Buckwald RA, Soenksen D, Malik Z (1996) Spectral bio-imaging. In: Fluorescence Imaging Spectroscopy and Microscopy. Wang XF and Herman B (eds) John Wiley & Sons, Inc.

Garini, Y., Macville, M., du Manoir, S., Buckwald, R.A., Lavi, M., Katzir, N., Wine, D., Bar-Am, I., Schröck, E., Cabib, D., Ried, T. Spectral karyotyping. Bioimaging (in press).

Gall JG, Pardue ML (1969) Formation and detection of RNA-DNA hybrid molecules in cytological preparations. Proc Natl Acad Sci USA 63:378-383.

Gray JW, Lucas JN, Pinkel D, Awa A (1992) Structural chromosome analysis by whole chromosome painting for assessment of radiation-induced genetic damage. J Radiat Res. 33:80-86.

Guan X-Y, Meltzer PS, Trent J (1994) Rapid generation of whole chromosome painting probes (WCPs) by chromosome microdissection. Genomics 22:101-107.

Guttenbach M, Schmid M (1991) Non-isotopic detection of chromosome 1 in human meiosis and demonstration of disomic sperm nuclei. Hum Genet 87:261-265.

Haaf T, Schmid M (1989) Centromeric association and non random distribution of centromeres in human tumour cells. Hum Genet 81:137-143.

Heiskanen M, Peltonen L, Palotif A. Visual mapping by high resolution FISH. Trends Genet 1996, 12:379-82

Heng HHQ, Squire J, Tsui L-C (1992) High resolution mapping of mammalian genes by in situ hybridization to free chromatin. Proc Natl Acad Sci USA 89:9509-9513.

Heselmeyer K, Schröck E, du Manoir S, Blegen H, Shah K, Steinbeck R, Auer G, Ried T. (1996) Gain of chromosome 3q defines the transition from severe dysplasia to invasive carcinoma of the uterine cervix. Proc Natl Acad Sci USA 93:484-497.

Hiraoka Y, Sedat JW, Agard DA (1987) The use of a charge-coupled device for quantitative optical microscopy of biological structures. Science 238:36-41.

Holmquist GP (1992) Chromosome bands, their chromatin flavors, and their functional features. Am J Hum Genet 51:17-37.

Huang S, Spector DL (1991) Nascent pre-mRNA transcripts are associated with nuclear regions enriched in splicing factors. Genes Dev 5:2288-2302.

Jauch A, Daumer C, Lichter P, Murken J, Schroeder-Kurth T, Cremer T (1990) Chromosomal in situ suppression hybridization of human gonosomes and autosomes and its use in clinical cytogenetics. Hum Genet 85:145-150.

Jauch A, Wienberg J, Stanyon R, Arnold N, Tofanelli S, Ishida T, Cremer T (1992) Interchromosomal rearrangements during hominoid evolution (human, great apes, gibbons) reconstructed by chromosomal in situ suppression hybridization. Proc Natl Acad Sci USA 89:8611-8615.

Johnson CV, McNeil JA, Carter KC, Lawrence JB (1991) A simple, rapid technique for precise mapping of multiple sequences in two colors using a single optical filter set. Genet Anal Techn Appl 8:24-35.

Kallioniemi A, Kallioniemi O-P, Sudar D, Rutovitz D, Gray JW, Waldman F, Pinkel D (1992) Comparative genomic hybrization for molecular cytogenetic analysis of solid tumors. Science 258:818-821.

Kallioniemi O-P, Kallioniemi A, Sudar D, Rutovitz D, Gray JW, Waldman F, Pinkel D (1993) Comparative genomic hybridization: a rapid new method for detecting and mapping DNA amplification in tumors. Sem Cancer Biol 4:41-46.

Kitsberg D, Selig S, Brandeis M, Simon I, Keshet I, Driscoll DJ, Nicholls RD, Cedar H (1993) Allele-specific replication timing of imprinted gene regions. Nature 364:459-463.

Klever M, Grond-Ginsbach C, Scherthan H, Schroeder-Kurth T (1991) Chromosomal in situ suppression hybridization after Giemsa banding. Hum Genet 86:484-486.

Klever M, Grond-Ginsbach CJ, Hager HD, Schroeder-Kurth TM (1992) Chorionic villus metaphase chromosomes and interphase nuclei analyzed by chromosomal in situ suppression (CISS) hybridization. Prenat Diagn 12:53-59.

Klinger KW, Landes G, Shook D, Harvey R, Lopez L, Locke P, Lerner T, Osathanondh R, Leverine B, Houseal T, Pavelka K, Dackowski W (1992) Rapid detection of chromosome aneuploidies in uncultured amniocytes by using fluorescence in situ hybridization (FISH). Am J Hum Genet 51:55-65.

Knuutila S, Bjorkqvist AM, Autio K, Tarkkanen M, Wolf M, Monni O, Szymanska J, Larramendy ML, Tapper J, Pere H, El-Rifai W, Hemmer S, Wasenius VM, Vidgren V, Zhu Y (1998) DNA copy number amplifications in human neoplasms: review of comparative genomic hybridization
studies. Am J Pathol;152:1107-23

Korenberg JR, Rykowski MC (1988) Human genome organization: Alu, Lines and the molecular structure of metaphase chromosome bands. Cell 53:391-400.

Landegent JE, Jansen in de Wal N, Dirks RW, Baas F, van der Ploeg M (1987) Use of whole cosmid cloned genomic sequences for chromosomal localization by non-radioactive in situ hybridization. Hum Genet 77:366-370.

Lawrence JB, Singer RH, Marselle LM (1989) Highly localized tracks of specific transcripts within interphase nuclei visualized by in situ hybridization. Cell 57:493-502.

Lawrence JB, Singer RH, McNeil JA (1990) Interphase and metaphase resolution of different distances within the dystrophin gene. Science 249:928-932.

Ledbetter DH (1992a) Cryptic translocations and telomere integrity. Am J Hum Genet 51:451-456.

Lengauer C, Riethman H, Cremer T (1990) Painting of human chromosomes with probes generated from hybrid cell lines by PCR with Alu and L1 primers. Hum Genet 86:1-6.

Lengauer C, Green ED, Cremer T (1992) Fluorescence in situ hybridization of YAC clones after Alu-PCR amplification. Genomics 13:826-828.

Lengauer C, Speicher MR, Popp S, Jauch A, Taniwaki M, Nagaraja R, Riethman HC, Donis-Keller H, D'Urso M, Schlessinger D, Cremer T (1993) Chromosomal bar codes produced by multicolor fluorescence in situ hybridization with multiple YAC clones and whole chromosome painting probes. Hum Mol Genet 2:505-512.

Lichter P, Cremer T, Borden J, Manuelidis L, Ward DC (1988) Delineation of individual human chromosomes in metaphase and interphase cells by in situ suppression hybridization using chromosome specific library probes.

Hum Genet 80:224-234.

Lichter P, Chang Tang C-J, Call K, Hermanson G, Evans GA, Housman D, Ward DC (1990) High resolution mapping of human chromosome 11 by in situ hybridization with cosmid clones. Science 247:64-69.

Liyanage, M., Coleman, A., du Manoir, S., Veldman, T., McCormack, S., Dickson, R.B., Barlow, C., Wynshaw-Boris, A., Janz, S., Wienberg, J., Ferguson-Smith, M.A., Schröck, E., Ried, T. (1996) Multicolor spectral karyotyping of mouse chromosomes. Nature Genet. 14:312-315.

Macville, M., Schröck, E., Padilla-Nash, H., Keck, C., Ghadimi, B.M., Zimonjic, D., Popescu, N., Ried, T. (1999) Comprehensive and definitive molecular cytogenetic characterization of Hela cells by spectral karyotyping. Cancer Res. 59: 141-150.

Malik Z, Cabib D, Buckwald RA, Talmi A, Garini Y, Lipson SG (1996) Fourier Transform multipixel spectroscopy for quantitative cytology. J. Microscopy 182, 133-140.

Manuelidis L (1984) Different central nervous system cell types display distinct and nonrandom arrangement of satellite DNA sequences. Proc Natl Acad Sci USA 81:3123-3127.

Manuelidis L (1985) Individual interphase chromosome domains revealed by in situ hybridization. Hum Genet 71:288-293.

Marcus DA (1988) High-performance optical filters for fluorescence analysis. Cell Motil Cytoskelet 10:62-70.

Meltzer PS, Guan X-Y, Burgess A, Trent JM (1992) Rapid generation of region specific probes by chromosome microdissection and their application. Nature Genet 1:24-28.

Michalet X, Ekong R, Fougerousse F, Rousseaux S, Schurra C, Hornigold N, van Slegtenhorst M, Wolfe J, Povey S, Beckmann JS, Bensimon A. Dynamic molecular combing: stretching the whole human genome for high-resolution studies. Science 1997 ,277:1518-23

Nederlof P, van der Flier S, Raap AK, Tanke HJ, van der Ploeg M, Kornips F, Geraedts JPM (1989) Detection of chromosome aberrations in interphase tumor nuclei by nonradioactive in situ hybridization. Cancer Genet Cytogenet 42:87-98.

Nederlof P, van der Flier S, Wiegant J, Raap AK, Tanke HJ, Ploem JS, van der Ploeg M (1990) Multiple fluorescence in situ hybridization. Cytometry 11:126-131.

Nelson DL, Ledbetter SA, Corbo L, Victoria MF, Ramirez-Solis, R, Webster T, Ledbetter DH, Caskey CT (1989) Alu polymerase chain reaction: A method for rapid isolation of human-specific sequences from complex DNA sources. Proc Natl Acad Sci USA 86:6686-6690.

Parra I, Windle B (1993) High resolution visual mapping of stretched DNA by fluorescent hybridization. Nature Genet 5:17-21.

Pieters MHEC, Geraedts JPM, Meyer H, Dumoulin JCM, Evers JLH, Jongbloed RJE, Nederlof PM, van der Flier S (1990) Human gametes and zygotes studies by nonradioactive in situ hybridization. Cytogenet Cell Genet 53:15-19.

Pinkel D, Straune T, Gray JW (1986) Cytogenetic analysis using quantitative, high sensitivity, fluorescence hybridization. Proc Natl Acad Sci USA 83:2934-2938.

Pinkel D, Landegent J, Collins C, Fuscoe J, Segraves R, Lucas J, Gray JW (1988) Fluorescence in situ hybridization with human chromosome specific libraries: detection of trisomy 21 and translocation of chromosome 4. Proc Natl Acad Sci USA 85:9138-9142.

Ploem JS, Tanke HJ (1987) Introduction to fluorescence microscopy. In: RMS microscopy handbooks series No. 10, Oxford Science Publications.

Popp S, Scholl HP, Loos P, Jauch A, Stelzer E, Cremer C, Cremer T (1990) Distribution of chromosome 18 and X centric heterochromatin in the interphase nucleus of cultured human cells. Exp Cell Res 189:1-12.

Popp S, Cremer T (1992) A biological dosimeter based on translocation scoring after multicolor CISS hybridization of chromosomal subsets. J Radiat Res 33:61-70.

Popp S, Jauch A, Schindler D, Speicher MR, Lengauer C, Donis-Keller H, Riethman HC, Cremer T (1993) Identification of a small, unbalanced translocation by multicolor fluorescence in situ hybridization. Hum Genet 92:527-532.

Ried T, Mahler V, Vogt P, Blonden L, van Ommen GJB, Cremer T, Cremer M (1990) Direct carrier detection by in situ suppression hybridization with cosmid clones for the Duchenne/Becker muscular dystrophy locus. Hum Genet 85:581-586.

Ried T, Baldini A, Rand TC, Ward DC (1992a) Simultaneous visualization of seven different DNA probes using combinatorial fluorescence and digital imaging microscopy. Proc Natl Acad Sci USA 89:1388-1392.

Ried T, Landes G, Dackowski W, Klinger K, Ward DC (1992b) Multicolor fluorescence in situ hybridization for the simultaneous detection of probe sets for chromosomes 13, 18, 21, X and Y in uncultured amniotic fluid cells. Hum Mol Genet 1:307-313.

Ried T, Lengauer C, Cremer T, Wiegant J, Raap AK, van der Ploeg M, Groitl P, Lipp M (1992c) Specific metaphase and interphase detection of the breakpoint region in 8q24 of Burkitt lymphoma cells by triple-color fluorescence in situ hybridization. Genes Chrom Cancer 4:69-74.

Ried T, Arnold N, Ward DC, Wienberg J (1993a) Comparative high-resolution mapping of human and primate chromosomes by fluorescence in situ hybridization. Genomics 18:381-386.

Ried T, Lengauer C, Lipp M, Fischer C, Cremer T, Ward DC (1993b) Evaluation of the utility of interphase cytogenetics to detect residual cells with a malignant genotype in mixed cell populations: a Burkitt lymphoma model. DNA Cell Biol 12:637-643.

Ried T, Rudy B, Vega-Saenz de Miera E, Lau D, Ward DC, Sen K (1993c) Localization of a highly conserved human potassium channel gene (NGK2-KV4;KCNC1) to chromosome 11p15. Genomics 15:405-411

Ried T, Petersen I, Holtgreve-Grez, Speicher MR, Schröck E, du Manoir S, Cremer T. (1994) Mapping of multiple DNA gains and losses in primary small cell lunc carcinomas by comparative genomic hybridization. Cancer Res 54:1801-1806.

Ried T, Just KE, Holtgreve-Grez H, du Manoir S, Speicher MR, Schröck E, Latham C, Blegen H, Zetterberg A, Cremer T, Auer G. (1995) Comparative genomic hybridization reveals a different pattern of chromosomal gains and losses in fibroadenomas and diploid and aneuploid breast carcinomas. Cancer Res 55:5415-5423.

Ried, T., Knutzen, R., Steinbeck, R., Blegen, H., Schröck, E., Heselmeyer, K., du Manoir, S., and Auer, G. (1996).

Comparative genomic hybridization reveals a specific pattern of chromosomal gains and losses during the genesis of colorectal tumors. Genes Chromosom. Cancer 15:234-245.

Ried, T., Liyanage, M., du Manoir, S., Heselmeyer, K., Auer, G., Macville, M., and Schröck, E. (1997) Tumor cytogenetics revisited: comparative genomic hybridization and spectral karyotyping. J. Mol. Med. 75:801-814.

Sawyer JR, Lukacs JL, Munshi N, Desikan KR, Singhal S, Mehta J, Siegel D, Shaughnessy J, Barlogie B. Identification of new nonrandom translocations in multiple myeloma with multicolor spectral karyotyping. Blood 1998; 92:4269-78

Schardin M, Cremer T, Hager HD, Lang M (1985) Specific staining of human chromosomes in chinese hamster x man hybrid cell lines demonstrates interphase chromosome territories. Hum Genet 71:281-287.

Schröck E, Thiel G, Lozanova T, du Manoir S, Meffert M-C, Jauch A, Speicher MR, Nürnberg P, Vogel S, Jänisch W, Donis-Keller H, Ried T, Witkowski R, Cremer T. (1994) Comparative genomic hybridization of human malignant gliomas reveals multiple amplification sites and non-random chromosomal gains and losses. Am J Pathol 144:1203-1218.

Schröck E, Blume, C., Meffert, M.C., du Manoir, S., Bersch, W., Kiessling, M., Lozanowa, T., Thiel, G., Witkowski, R., Ried, T., Cremer, T. (1996) Recurrent gain of chromosome 7q in low grade astrocytic tumors studied by comparative genomic hybridization. Genes Chromosom. Cancer 15:199-205.

Schröck, E., du Manoir, S., Veldman, T., Schoell, B., Wienberg, J., Ferguson-Smith, M.A., Ning, Y., Ledbetter, D.H., Soenksen, D., Garini, Y., Ried, T. (1996) Multicolor spectral karyotyping of human chromosomes. Science 273:494-497.

Schröck, E., Veldman, T., Padilla-Nash, H., Ning, Y., Spurbeck, J., Jalal, S., Schaffer, L.G., Papenhausen, P., Kozma, C., Phelan, M.C., Kijeldsen, E., Schonberg, S.A., Biesecker, L., du Manoir, S., Ried, T. (1997) Spectral karyotyping refines cytogenetic diagnostics of constitutional chromosomal abnormalities. Hum. Genet. 101:255-262.

Selig S, Okumura K, Ward DC, Cedar H (1992) Delineation of DNA replication time zones by fluorescence in situ hybridization. EMBO J 11:1217-1225.

Speicher MR, du Manoir S, Schröck E, Holtgreve-Grez H, Schoell B, Lengauer C, Cremer T, Ried T (1993) Molecular cytogenetic analysis of formalin fixed, paraffin embedded solid tumors by comparative genomic hybridization after universal DNA amplification. Hum Mol Genet 2:1907-1914.

Speicher MR, Jauch A, Jochum W, du Manoir S, Ried T, Walt H, Cremer T. (1995) Correlation of microscopic phenotype with genotype in a formalin fixed, paraffin embedded testis tumor using universal DNA amplification, comparative genomic hybridization and interphase cytogenetics. Am J Pathol 146:1332-1340.

Speicher M, Ballard SG, Ward DC (1996) Karyotyping human chromosomes by combinatorial multi-fluor FISH. Nature Genet 12:368-375

Spector DL (1990) Higher order nuclear organization: Three dimensional distribution of small nuclear ribonucleoprotein particles. Proc. Natl. Acad. Sci. 87:147-151.

Spector DL, Fu X-D, Maniatis T (1991) Association between distinct pre-mRNA splicing components and the cell

nucleus. EMBO J. 10:3467-3481.

Stilgenbauer S, Döhner H, Bulgary-Mörschel M, Weitz S, Bentz M, Lichter P (1993) Retinoblastoma gene deletion in chronic lymphoid leukemias: a combined metaphase and interphase cytogenetic study. Blood 81:2118-2124.

Tanke HJ (1989) Does light microscopy have a future? J Microsc 155:405-418.

Telenius H, Pelear AH, Tunnacliffe A, Carter NP, Behmel A, Ferguson-Smith MA, Norednskjöld M, Pfragner R, Ponder BAJ (1992) Cytogenetic analysis by chromosome painting using DOP-PCR amplified flow sorted chromosomes. Genes Chrom Cancer 4:267-263.

Telenius H, Carter NP, Bebb CE, Nordenskjöld M, Ponder BAJ, Tunnacliffe A (1992) Degenerate oligonucleotide primed PCR: general amplification of target DNA by a single degenerate primer. Genomics 13:718-725.

Tkachuk DC, Pinkel D, Kuo W-L, Weier H-U, Gray JW (1991) Clinical applications of fluorescence in situ hybridization. Genet Anal Techn Appl 8:67-74.

Tocharoentanaphol C, Cremer M, Schröck E, Kilian K, Blonden L, Cremer T, Ried T (1994) Multicolor fluorescence in situ hybridization on metaphase chromosomes and interphase Halo-preparations using cosmid and YAC clones for the simultaneous high resolution mapping of deletions in the dystrophin gene. Hum Genet 93:229-235.

Trask BJ, Massa H, Kenwrick S, Gitschier J (1991) Mapping of human chromosome Xq28 by two-color fluorescence in situ hybridization of DNA sequences to interphase nuclei. Am J Hum Genet 48:1-15.

Van Dekken H, van der Voort HTM, Brakenhoff GJ, Bauman JGJ (1990) Three dimensional reconstruction of pericentric DNA and ribosomal RNA sequences in HL60 cells after double target in situ hybridization and confocal microscopy. Cytometry 11:579-585.

Veldman, T., Vignon, C., Schröck, E., Rowley, J.D., and Ried, T. (1997) Hidden chromosomal abnormalities in hematological malignancies detected by multicolor spectral karyotyping. Nature Genet. 15:406-410.

Verma RS, Babu A (1989). Human chromosomes: manual of basic techniques. Pergamon Press, Elmsford.

Vogt P (1990) Potential genetic functions of tandem repeated DNA sequence blocks in the human genome are based on a highly conserved “chromatin folding code”. Hum Genet 84:301-336.

Ward BE, Gersen SL, Carelli MP, McGuire NM, Dackowski WR, Weinstein M, Sandlin C, Warren R, Klinger KW (1993) Rapid prenatal diagnosis of chromosomal aneuploidies by fluorescence in situ hybridization: clinical experience with 4500 specimens. Am J Hum Genet 52:854-865.

Ward DC, Lichter P, Boyle A, Baldini A, Menninger J, Ballard SG (1991) Gene mapping by fluorescent in situ hybridization and digital imaging microscopy. In: Etiology of human diseases at the DNA level. Lindsten J, Petterson U (eds). Raven Press. New York.

Weier HU, Wang M, Mullikin JC, Zhu Y, Cheng JF, Greulich KM, Bensimon A, Gray JW. Quantitative DNA fiber mapping. Hum Mol Genet 1995, 4:1903-10

Weimer R, Haaf T, Krüger J, Poot M, Schmid M (1992) Characterization of centromere arrangements and test for random distribution in G_0, G_1, G_2, G_0, and early S phase in human lymphocytes. Hum Genet 88:673-682.

Wiegant J, Ried T, Nederlof P, van der Ploeg M, Tanke HJ, Raap AK (1991) In situ hybridization with fluoresceinated DNA. Nucl Acids Res 19:3237-3241.

Wiegant J, Kalle W, Mullenders L, Brookes S, Hoovers JMN, Dauwerse JG, van Ommen GJB, Raap AK (1992) High-resolution in situ hybridization using DNA halo preparations. Hum Mol Genet 1:587-591.

Wiegant J, Wiesmeijer CC, Hoovers JMN, Schuuring E, d'Azzo A, Vrolijk J, Tanke HJ, Raap AK (1993) Multiple and sensitive fluorescence in situ hybridization with rhodamine-, fluorescein-, and coumarin labeled DNAs. Cytogenet Cell Genet 63:73-76.

Wienberg J, Jauch A, Stanyon R, Cremer T (1990) Molecular cytotaxonomy of primates by chromosomal in situ suppression hybridization. Genomics 8:347-270.

Wienberg J, Stanyon R, Jauch A, Cremer T (1992) Homologies in human and Macaca fuscata chromosomes revealed by in situ suppresion hybridization with human chromosome specific DNA libraries. Chromosoma 101:265-270.

Willard HF, Waye JS (1987) Hierarchical order in chromosome-specific human alpha satellite DNA. Trends Genet 3:192-198.

Xing Y, Johnson CV, Dobner PR, Lawrence JB (1993) Higher level organization of individual gene transcription and RNA splicing. Science 259:1326-1330.

Zirbel RM, Mathieu UR, Kurz A, Cremer T, Lichter P (1993) Evidence for a nuclear compartment of transcription and splicing located at chromosome domain boundaries. Chromosome Res 1:93-106.